

SEQUENCE LISTING

<110> Japan Tobacco, Inc.

<120> HCV Polymerase Suitable For Crystal Structure Analysis
And Method For Using The Enzyme

<130> J00-0086

<140>

<141>

<150> JP P1999-188630

<151> 1999-07-02

<150> JP P1999-192488

<151> 1999-07-07

<160> 24

<170> PatentIn Ver. 2.1

<210> 1

<211> 591

<212> PRT

<213> Hepatitis C virus

<400> 1

Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Ile Thr Pro Cys Ala Ala

1	5	10	15
Glu Glu Ser Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu Arg			
	20	25	30
His His Asn Met Val Tyr Ala Thr Thr Ser Arg Ser Ala Gly Leu Arg			
	35	40	45
Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Asp His Tyr			
	50	55	60
Arg Asp Val Leu Lys Glu Met Lys Ala Lys Ala Ser Thr Val Lys Ala			
	65	70	75
Lys Leu Leu Ser Val Glu Glu Ala Cys Lys Leu Thr Pro Pro His Ser			
	85	90	95
Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Asn Leu Ser			
	100	105	110
Ser Lys Ala Val Asn His Ile His Ser Val Trp Lys Asp Leu Leu Glu			
	115	120	125
Asp Thr Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu Val			
	130	135	140
Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu Ile			
	145	150	155
			160

Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu Tyr
 165 170 175

Asp Val Val Ser Thr Leu Pro Gln Val Val Met Gly Ser Ser Tyr Gly
 180 185 190

Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Asn Thr Trp
 195 200 205

Lys Ser Lys Lys Asn Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe
 210 215 220

Asp Ser Thr Val Thr Glu Asn Asp Ile Arg Val Glu Glu Ser Ile Tyr
 225 230 235 240

Gln Cys Cys Asp Leu Ala Pro Glu Ala Arg Gln Ala Ile Lys Ser Leu
 245 250 255

Thr Glu Arg Leu Tyr Ile Gly Gly Pro Leu Thr Asn Ser Lys Gly Gln
 260 265 270

Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr Ser
 275 280 285

Cys Gly Asn Thr Leu Thr Cys Tyr Leu Lys Ala Ser Ala Ala Cys Arg
 290 295 300

Ala Ala Lys Leu Gln Asp Cys Thr Met Leu Val Asn Gly Asp Asp Leu
 305 310 315 320

Val Val Ile Cys Glu Ser Ala Gly Thr Gln Glu Asp Ala Ala Ser Leu
 325 330 335

Arg Val Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp
 340 345 350

Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser
 355 360 365

Asn Val Ser Val Ala His Asp Ala Ser Gly Lys Arg Val Tyr Tyr Leu
 370 375 380

Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala
 385 390 395 400

Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Tyr Ala
 405 410 415

Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser Ile
 420 425 430

Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln Ile Tyr
 435 440 445

Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile Ile Glu
 450 455 460

Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly

465	470	475	480
Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val Pro Pro			
	485	490	495
Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu Leu			
	500	505	510
Ser Gln Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe Asn Trp			
	515	520	525
Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala Ser Gln			
	530	535	540
Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly Asp Ile			
545	550	555	560
Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Trp Phe Met Leu Cys Leu			
	565	570	575
Leu Leu Leu Ser Val Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg			
	580	585	590

<210> 2

<211> 1743

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:DNA encoding
 fusion protein consisting of a portion of HCV
 polymerase and histidine tag at the C-terminus

<220>

<221> CDS

<222> (1)..(1743)

<400> 2

atg tca atg tcc tac aca tgg aca ggc gcc ttg atc acg cca tgc gct 48

Met Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Ile Thr Pro Cys Ala

1

5

10

15

gcg gag gaa agc aag ctg ccc atc aac gcg ttg agc aac tct ttg ctg 96

Ala Glu Glu Ser Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu

20

25

30

cgc cac cat aac atg gtt tat gcc aca aca tct cgc agc gca ggc ctg 144

Arg His His Asn Met Val Tyr Ala Thr Thr Ser Arg Ser Ala Gly Leu

35

40

45

cgg cag aag aag gtc acc ttt gac aga ctg caa gtc ctg gac gac cac 192

Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Asp His

50

55

60

tac cgg gac gtg ctc aag gag atg aag gcg aag gcg tcc aca gtt aag 240

Tyr Arg Asp Val Leu Lys Glu Met Lys Ala Lys Ala Ser Thr Val Lys

65

70

75

80

gct aaa ctc cta tcc gta gag gaa gcc tgc aag ctg acg ccc cca cat 288
 Ala Lys Leu Leu Ser Val Glu Glu Ala Cys Lys Leu Thr Pro Pro His

85

90

95

tcg gcc aaa tcc aag ttt ggc tat ggg gca aag gac gtc cgg aac cta 336
 Ser Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Asn Leu

100

105

110

tcc agc aag gcc gtt aac cac atc cac tcc gtg tgg aag gac ttg ctg 384
 Ser Ser Lys Ala Val Asn His Ile His Ser Val Trp Lys Asp Leu Leu

115

120

125

gaa gac act gtg aca cca att gac acc acc atc atg gca aaa aat gag 432
 Glu Asp Thr Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu

130

135

140

gtt ttc tgt gtc caa cca gag aaa gga ggc cgt aag cca gcc cgc ctt 480
 Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu

145

150

155

160

atc gta ttc cca gat ctg gga gtc cgt gta tgc gag aag atg gcc ctc 528
 Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu

165

170

175

tat gat gtg gtc tcc acc ctt cct cag gtc gtg atg ggc tcc tca tac 576
 Tyr Asp Val Val Ser Thr Leu Pro Gln Val Val Met Gly Ser Ser Tyr

180

185

190

gga ttc cag tac tct cct ggg cag cga gtc gag ttc ctg gtg aat acc 624
 Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Asn Thr
 195 200 205

tgg aaa tca aag aaa aac ccc atg ggc ttt tca tat gac act cgc tgt 672
 Trp Lys Ser Lys Lys Asn Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys
 210 215 220

ttc gac tca acg gtc acc gag aac gac atc cgt gtt gag gag tca att 720
 Phe Asp Ser Thr Val Thr Glu Asn Asp Ile Arg Val Glu Glu Ser Ile
 225 230 235 240

tac caa tgt tgt gac ttg gcc ccc gaa gcc aga cag gcc ata aaa tcg 768
 Tyr Gln Cys Cys Asp Leu Ala Pro Glu Ala Arg Gln Ala Ile Lys Ser
 245 250 255

ctc aca gag cgg ctt tat atc ggg ggt cct ctg act aat tca aaa ggg 816
 Leu Thr Glu Arg Leu Tyr Ile Gly Gly Pro Leu Thr Asn Ser Lys Gly
 260 265 270

cag aac tgc ggt tat cgc cgg tgc cgc gcg agc ggc gtg ctg acg act 864
 Gln Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr
 275 280 285

agc tgc ggt aac acc ctc aca tgt tac ttg aag gcc tct gca gcc tgt 912
 Ser Cys Gly Asn Thr Leu Thr Cys Tyr Leu Lys Ala Ser Ala Ala Cys
 290 295 300

cga gct gcg aag ctc cag gac tgc acg atg ctc gtg aac gga gac gac 960

Arg Ala Ala Lys Leu Gln Asp Cys Thr Met Leu Val Asn Gly Asp Asp
 305 310 315 320

ctc gtc gtt atc tgt gaa agc gcg gga acc caa gag gac gcg gcg agc 1008
 Leu Val Val Ile Cys Glu Ser Ala Gly Thr Gln Glu Asp Ala Ala Ser
 325 330 335

cta cga gtc ttc acg gag gct atg act agg tac tcc gcc ccc ccc ggg 1056
 Leu Arg Val Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly
 340 345 350

gac ccg ccc caa cca gaa tac gac ttg gag ctg ata aca tca tgt tcc 1104
 Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser
 355 360 365

tcc aat gtg tcg gtc gcc cac gat gca tca ggc aaa agg gtg tac tac 1152
 Ser Asn Val Ser Val Ala His Asp Ala Ser Gly Lys Arg Val Tyr Tyr
 370 375 380

ctc acc cgt gat ccc acc acc ccc ctc gca cgg gct gcg tgg gag aca 1200
 Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr
 385 390 395 400

gct aga cac act cca gtt aac tcc tgg cta ggc aac att att atg tat 1248
 Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Tyr
 405 410 415

gcg ccc act ttg tgg gca agg atg att ctg atg act cac ttc ttc tcc 1296
 Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser

420	425	430	
atc ctt cta gcg cag gag caa ctt gaa aaa gcc ctg gac tgc cag atc			1344
Ile Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln Ile			
435	440	445	
tac ggg gcc tgt tac tcc att gag cca ctt gac cta cct cag atc att			1392
Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile Ile			
450	455	460	
gaa cga ctc cat ggc ctt agc gca ttt tca ctc cat agt tac tct cca			1440
Glu Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro			
465	470	475	480
ggc gag atc aat agg gtg gct tca tgc ctc agg aaa ctt ggg gta cca			1488
Gly Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val Pro			
485	490	495	
ccc ttg cga gtc tgg aga cat cgg gcc agg agc gtc cgc gct agg cta			1536
Pro Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu			
500	505	510	
ctg tcc cag ggg ggg agg gcc gcc act tgt ggc aag tac ctc ttc aac			1584
Leu Ser Gln Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe Asn			
515	520	525	
tgg gca gtg aag acc aaa ctc aaa ctc act cca atc ccg gct gcg tcc			1632
Trp Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala Ser			
530	535	540	

cag ctg gac ttg tcc ggc tgg ttc gtt gct ggt tac agc ggg gga gac 1680
 Gln Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly Asp
 545 550 555 560

ata tat cac agc ctg tct cgt gcc cga ccc cgc gga tcc cat cac cat 1728
 Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Gly Ser His His His
 565 570 575

cac cat cac taa taa 1743
 His His His
 580

<210> 3

<211> 579

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence:DNA encoding
 fusion protein consisting of a portion of HCV
 polymerase and histidine tag at the C-terminus

<400> 3

Met Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Ile Thr Pro Cys Ala
 1 5 10 15
 Ala Glu Glu Ser Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu
 20 25 30
 Arg His His Asn Met Val Tyr Ala Thr Thr Ser Arg Ser Ala Gly Leu
 35 40 45

Arg Gln Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Asp His
 50 55 60
 Tyr Arg Asp Val Leu Lys Glu Met Lys Ala Lys Ala Ser Thr Val Lys
 65 70 75 80
 Ala Lys Leu Leu Ser Val Glu Glu Ala Cys Lys Leu Thr Pro Pro His
 85 90 95
 Ser Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Asn Leu
 100 105 110
 Ser Ser Lys Ala Val Asn His Ile His Ser Val Trp Lys Asp Leu Leu
 115 120 125
 Glu Asp Thr Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu
 130 135 140
 Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu
 145 150 155 160
 Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu
 165 170 175
 Tyr Asp Val Val Ser Thr Leu Pro Gln Val Val Met Gly Ser Ser Tyr
 180 185 190
 Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Asn Thr
 195 200 205
 Trp Lys Ser Lys Lys Asn Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys
 210 215 220
 Phe Asp Ser Thr Val Thr Glu Asn Asp Ile Arg Val Glu Glu Ser Ile
 225 230 235 240
 Tyr Gln Cys Cys Asp Leu Ala Pro Glu Ala Arg Gln Ala Ile Lys Ser
 245 250 255
 Leu Thr Glu Arg Leu Tyr Ile Gly Gly Pro Leu Thr Asn Ser Lys Gly
 260 265 270
 Gln Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr

275	280	285
Ser Cys Gly Asn Thr Leu Thr Cys Tyr Leu Lys Ala Ser Ala Ala Cys		
290	295	300
Arg Ala Ala Lys Leu Gln Asp Cys Thr Met Leu Val Asn Gly Asp Asp		
305	310	315
Leu Val Val Ile Cys Glu Ser Ala Gly Thr Gln Glu Asp Ala Ala Ser		
325	330	335
Leu Arg Val Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly		
340	345	350
Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser		
355	360	365
Ser Asn Val Ser Val Ala His Asp Ala Ser Gly Lys Arg Val Tyr Tyr		
370	375	380
Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr		
385	390	395
Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Tyr		
405	410	415
Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser		
420	425	430
Ile Leu Leu Ala Gln Glu Gln Leu Glu Lys Ala Leu Asp Cys Gln Ile		
435	440	445
Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Gln Ile Ile		
450	455	460
Glu Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro		
465	470	475
Gly Glu Ile Asn Arg Val Ala Ser Cys Leu Arg Lys Leu Gly Val Pro		
485	490	495
Pro Leu Arg Val Trp Arg His Arg Ala Arg Ser Val Arg Ala Arg Leu		
500	505	510

Leu Ser Gln Gly Gly Arg Ala Ala Thr Cys Gly Lys Tyr Leu Phe Asn
 515 520 525
 Trp Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Ile Pro Ala Ala Ser
 530 535 540
 Gln Leu Asp Leu Ser Gly Trp Phe Val Ala Gly Tyr Ser Gly Gly Asp
 545 550 555 560
 Ile Tyr His Ser Leu Ser Arg Ala Arg Pro Arg Gly Ser His His His
 565 570 575
 His His His

<210> 4

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
 synthesized primer sequence, 5BNde1FW.

<220>

<221> primer_bind

<222> (1)..(30)

<400> 4

catatgtcaa tgtcctacac atggacagcc

<210> 5

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
synthesized primer sequence, 5B570HRV.

<220>

<221> primer_bind

<222> (1)..(57)

<400> 5

ttattagtga tggatgatgt gatgggatcc gcggggtcgg gcacgagaca ggctgtg 57

<210> 6

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
synthesized primer sequence, 5B552HRV.

<220>

<221> primer_bind

<222> (1)..(57)

<400> 6

ttattagtga tggatgatgt gatgggatcc aacgaaccag cgggacaagt ccagctg 57

<210> 7

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
synthesized primer sequence, 5B544HRV.

<220>

<221> primer_bind

<222> (1)..(57)

<400> 7

ttattagtga tggatgatgt gatgggatcc ctgggacgca gccgggattg gaggtag 57

<210> 8

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially

synthesized primer sequence, 5B536HRV.

<220>

<221> primer_bind

<222> (1)..(67)

<400> 8

ttattagtga tggatgatggt gatgggatcc gagtttgagt ttggtcttca ctgccagtt 60
gaagagg 67

<210> 9

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
synthesized primer sequence, 5B531HRV.

<220>

<221> primer_bind

<222> (1)..(60)

<400> 9

ttattagtga tggatgatggt gatgggatcc cttcactgcc cagttgaaga ggtacttgcc 60

<210> 10

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Artificially
synthesized primer sequence, 5B591HRV.

<220>

<221> primer_bind

<222> (1)..(52)

<400> 10

ttattaatgg tgatggtgat ggtgtccgga tcgattgggg agcaggtaga tg 52

<210> 11

<211> 8

<212> PRT

<213> Hepatitis C virus

<400> 11

Xaa Xaa Xaa Leu Xaa Xaa Trp Phe Xaa

1

5

<210> 12

<211> 8

<212> PRT

<213> Hepatitis C virus

<400> 12

Lys Asp Leu Ser Gly Trp Phe Lys

1

5

<210> 13

<211> 9

<212> PRT

<213> Hepatitis C virus

<400> 13

Lys Lys Asp Leu Ser Gly Trp Phe Lys

1

5

<210> 14

<211> 8

<212> PRT

<213> Hepatitis C virus

<400> 14

Lys Asp Leu Ser Gly Trp Phe Val

1

5

<210> 15

<211> 8

<212> PRT

<213> Hepatitis C virus

<400> 15

Leu Asp Leu Ser Gly Trp Phe Lys

1

5

<210> 16

<211> 8

<212> PRT

<213> Hepatitis C virus

<400> 16

Leu Asp Leu Ser Gly Trp Phe Val

1

5

<210> 17

<211> 7

<212> PRT

<213> Hepatitis C virus

<400> 17

Asp Leu Ser Gly Trp Phe Val

1

5

<210> 18

<211> 6

<212> PRT

<213> Hepatitis C virus

<400> 18

Asp Leu Ser Gly Trp Phe

1

5

<210> 19

<211> 6

<212> PRT

<213> Hepatitis C virus

<400> 19

Leu Ser Gly Trp Phe Val

1

5

<210> 20

<211> 5

<212> PRT

<213> Hepatitis C virus

<400> 20

Leu Ser Gly Trp Phe

1

5

<210> 21

<211> 6

<212> PRT

<213> Hepatitis C virus

<400> 21

Leu Ser Gly Trp Phe Lys

1

5

<210> 22

<211> 6

<212> PRT

<213> Hepatitis C virus

<400> 22

Lys Leu Ser Gly Trp Phe

1

5

<210> 23

<211> 5

<212> PRT

<213> Hepatitis C virus

<400> 23

Leu Gly Gly Trp Phe

1

5

<210> 24

<211> 5

<212> PRT

<213> Hepatitis C virus

<400> 24

Leu Ser Asp Trp Phe

1

5